THESIS

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THE COLLOIDAL GOLD REACTION: A CONTRIBUTION TO SEROLOGY

R. G. Bannerman

M.A., M.B., Ch.B.,
(Hon. Capt., R.A.M.C.)

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FOREWORD

The Thesis is an attempt, in the first place, to assess the value of the Colloidal Gold reaction as a diagnostic procedure, and in the second place, to gain some insight into the nature of the reaction as a serological phenomenon.

In 1912, Lange, of Vienna, was making use of a colloidal gold solution in an effort to distinguish by means of the reaction obtained, between syphilitic and non-syphilitic sera --- but without success. Since then, however, the reaction with the cerebro-spinal fluid has been developed, especially in America. Put shortly, the test consists in mixing cerebro-spinal fluid in certain proportions with colloidal gold solution: with abnormal fluids there result colour and other changes more or less characteristic of some pathological conditions.

The reaction may now be said to take its place as a valuable adjunct in the laboratory diagnosis of disease of the central nervous system. The test has been little tried in this country, if one may judge by the paucity of publications on the subject. Those who have employed the test are unanimous in considering it a useful and reliable one, and the present series of results confirms that view.

Opportunities for the observations recorded here arose while the writer was pathologist to the Dykebar War Hospital, Paisley, in 1916 and 1917: in 1918, while pathologist to Robroyston War Hospital:

and more recently, in the Stephen Ralli Memorial Pathological Department attached to the Royal Sussex County Hospital, Brighton, some confirmatory work has been carried on. The first-mentioned of these hospitals dealt with mental cases, which supplied a long series of cerebrospinal fluids: these cases were chiefly sufferers from Dementia Paralytica (General Paralysis of the Insane), a disease in which the colloidal gold reaction is peculiarly characteristic. From time to time specimens were obtained from the Royal Infirmary, Edinburgh: these emanated chiefly from the wards of Dr. J.J.Graham Brown.

Much gratitude is felt towards the numerous colleagues who have helped in the supply of material, and afforded opportunities and encouragement in the laboratory.

The nature of the reaction has been very obscure, and a variety of experimental observations have been made side by side with the routine tests, with the object of elucidating the subject in some measure.

The matter is arranged as follows:—

(1) ---- TECHNIQUE of the TEST.

There are considerable technical difficulties in the preparation of suitable colloidal gold solution, which is the essential reagent in the test.

Numerous methods of preparation of colloidal gold have been practised, starting perhaps with the aurum potabile of the alchemists: Mme. Fulhame in 1794, experimented with such solutions, and the subject was much enlightened by the researches of Faraday, Berzelius, Davy, and Bredig: Faraday laid stress on the necessity for clean glass in all the manipulations. In 1898, Zsigmondy made an advance relevant to the present reaction when he elaborated a method for the preparation of colloidal gold solutions which consists essentially in the reduction of gold chloride in alkaline solution by formalin, or certain other organic reagents: for this procedure to succeed, a high standard of purity of distilled water has to be reached. Many other reagents than formalin have been used: (one of these which has been tried by the writer is hydrazin hydrate,* as used in Gutbier's method: (1)(2) with this method, the quantity of the reducing reagent and the alkalinity of the gold chloride solution determine the colour obtained: in the present experiments solutions of good colour were readily obtained, but were found to be quite unsuitable for the reaction: the reason of this failure is that in making gold solution by the hydrazin method, a considerable

* For a supply of this, the writer is indebted to Dr. W.W.Taylor.

degree of alkalinity has to be reached, so that when the solution comes to be neutralised for use in the test, the electrolyte content is so high as to precipitate the gold from its colloidal solution immediately. An attempt was then made to avoid the difficulty by using Ammonium hydrate in place of sodium hydrate, but this failed to give a solution. These solutions are not "protected" from the action of electrolytes and are easily prepared: it is unfortunate, therefore that they do not meet the requirements of the test.

Solutions thus made have an exceedingly low gold content --- about 0.005% of Au. As pointed out by Svedberg,(1) attempts to concentrate them by evaporation fail because the electrolytes in the solution reach such a concentration that the gold is precipitated from solution: but he used a parchment membrane to dialyse off the salts, and then succeeded in obtaining a 0.12% solution, with a correspondingly richer colour. This has been verified by the writer, using a collodion-impregnated filter paper dialyser: the former colour of the solution is restored on dilution with distilled water, and the solution is a very stable one: for practical purposes, however, the procedure was not considered to offer any advantages over what may be called the classical method.

The method suited to the purposes of the reaction is an elaboration of that of Zsigmondy, as described by Miller and his co-workers,(2),(3) whose papers form an invaluable starting-off place towards the practice of the test.

In the preparation of the reagent, the first requisite is new, and cleaned glass-ware, which has been immediately before use washed with water that has been thrice distilled in order to reach great purity. In a beaker thus cleaned, 1000 cc. of thrice distilled water is heated slowly until the temperature reaches 50 deg. The heating is then continued more rapidly until 60 deg. are reached and at that temperature, 10 cc. of 1% gold chloride are added, and 7 cc. of 2% potassium carbonate: at 80 deg., 10 drops of 1% Oxalic acid are added, the liquid being meanwhile stirred by the thermometer: at 90 deg., the source of heat is removed and 5 cc. of 1% formaldehyde* are added. The solution then, if satisfactory, acquires a pink colour which slowly develops into a brilliant orange-red. Before use the solution is to be neutralised if necessary, the neutral point being taken as the brown-red colour obtained on adding a few drops of alizarin red in 1% solution in 50% alcohol. Further, the solution must be non-protected, that is, the gold must be completely precipitated from 5 cc. of the colloidal solution by the addition of 1.7 cc. of 1% NaCl. And the solution must be perfectly clear. Reaction may be corrected to neutral, but a protected solution must be rejected.

This description is abstracted from the paper by Miller and his colleagues.[2],[3] While they insist on the use of Jena glassware, Merck's chemicals, and the avoidance of a rubber connection in the distilling

* 1 cc. 40% formaldehyde (formalin) in 40 cc. H_2O

apparatus, the writer's experience has been that a certain amount of latitude is permissible: thus, he has not been able to avoid a rubber or cork junction in the condenser, and Jena glass has not always been available: again, Merck's chemicals have been usually unobtainable, but the pure reagents on the British market have been found quite suitable. Johnson's gold chloride has been used throughout. Nevertheless, disappointing results have been met with from time to time, in spite of apparent faithfulness to all the requirements: as a general experience, however, a change of glassware is sufficient to remove the trouble, for the chief cause of failure — granted that the reagents are pure and freshly made up — is the formation of a colloidal silicate from the action of the distilled water on the glass: this either prevents the formation of the colloidal gold solution, or, permitting a solution of good colour to form, it exerts a protective action upon it, and so the solution is rendered useless. It may be noted, too, that a good gold solution has on several occasions been obtained when using water only twice distilled, but very freshly distilled.

It has been the usual experience that a solution satisfactory at the time of making is neutral, and remains so for several weeks if kept in the dark in a well-stoppered bottle.

Technique of the actual test: into the first of 11 clean test-tubes are placed I.8 cc. of freshly made, sterile 0.4% NaCl, and into the remainder 1 cc.

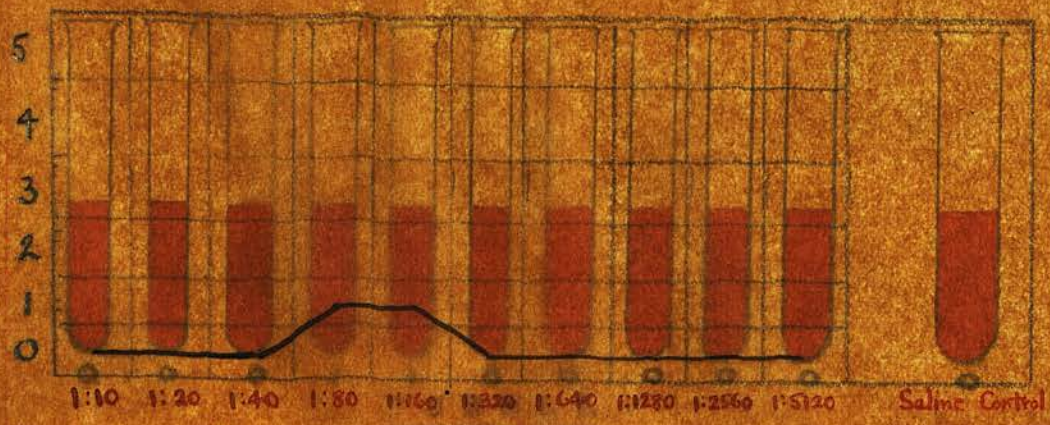
of the same solution: to the first tube there is now added 0.2 cc. of the spinal fluid to be tested: when this has been thoroughly mixed with the saline, I. cc. is pipetted off and placed in the second tube: I cc. of this mixture is removed in turn and placed in the third tube, and so on: on reaching the tenth tube, 1 cc. of the mixture is discarded: the eleventh tube, containing 1 cc. of 0.4% saline, is left as a control. A series of dilutions, commencing at 1:10, and extending through 1:20, 1:40, 1:80, 1:I60, 1:320, I:640, 1:I280, 1:2560, to 1:5I20, is thus set up. To each of these tubes are now added 5 cc. of the colloidal gold solution: the tubes are allowed to stand overnight and the result then read off: in the case of a normal fluid, there will be no change, or at most a tinge of lilac in the middle tubes: the result is therefore expressed, either graphically or as 0000000000, or perhaps, 0000II0000. But in the case of certain abnormal fluids, a striking change takes place, owing to varying degrees of "flocculation" of the colloidal gold, as expressed by alteration of colour: when the gold is completely precipitated, leaving a clear, colourless supernatant fluid, a number 5 is used to express the reaction: a pale-blue colour is designated a 4 reaction: a deeper blue is 3, a purple colour 2, and a red-blue I.

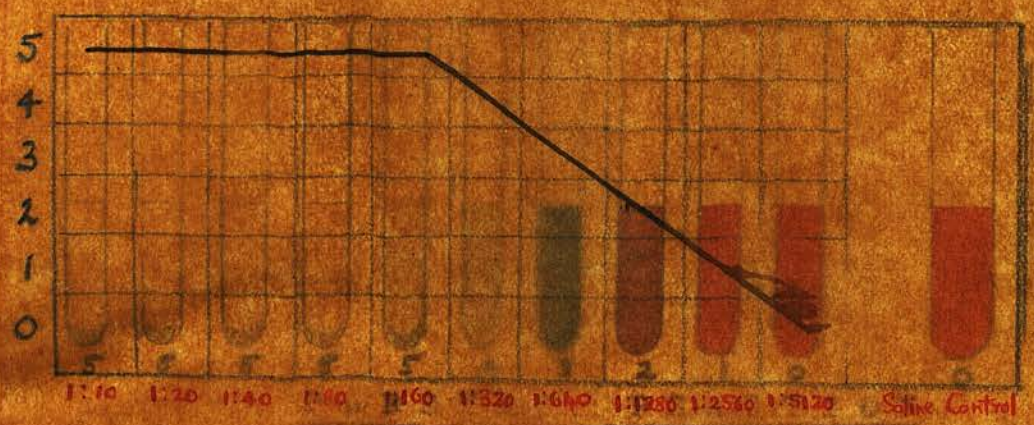
This description of technique is abstracted from the paper by Miller et al.[2],[3]

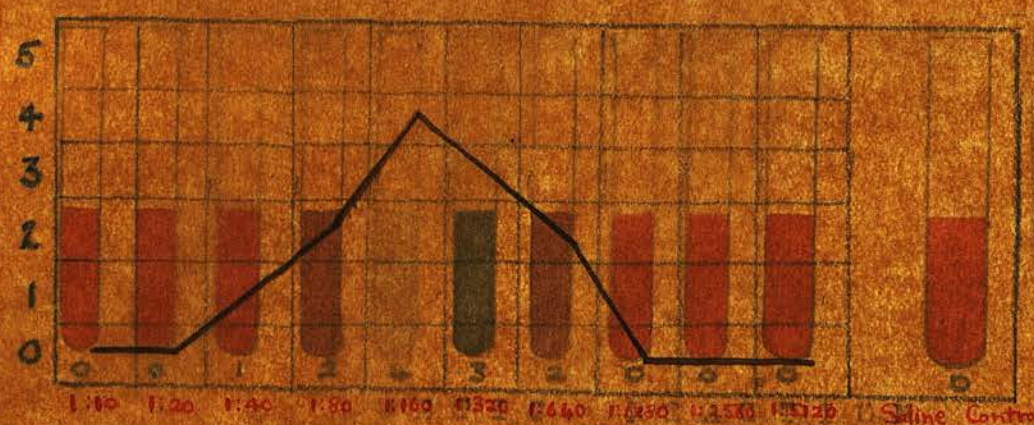
It is most convenient to express the result by a series of numbers: for example, a typical

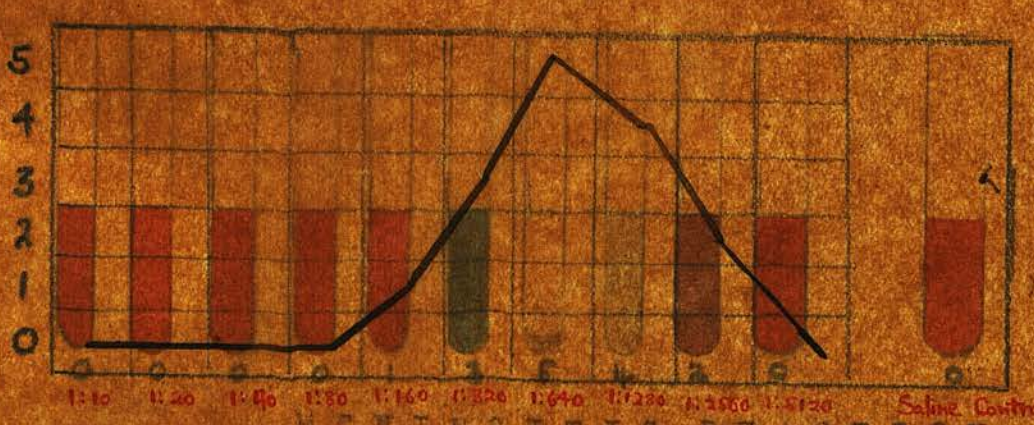
reading in a case of dementia paralytica (general paralysis of the insane) would be 5555543210, that is, there is complete precipitation of gold by about the first half of the series of dilutions. Another type of reaction is that in which there is no change visible in the earlier tubes of the series, but a colour change occurs in the higher dilutions: it is possible to distinguish two types of this kind of "curve": one, the "luetic", shows a maximum degree of flocculation at about the third or fourth tube: it is typically obtained in cases of syphilitic meningitis or gumma, or tabes: a luetic reaction is of the type 0012432000. The other type of reaction is the "meningitic": in this case, the maximum reaction occurs nearer the end of the series, thus:- 0000135420: this kind of curve is characteristically obtained in cases of infective meningitis, e.g., due to the B. Tuberculosis or the Meningococcus. In a single case, which is, so far as the writer knows, unique in its reaction, the curve was 0155555555.

The four common types of reaction are represented graphically on the next page, in relation to Plate I, which gives an indication of the colours obtained.

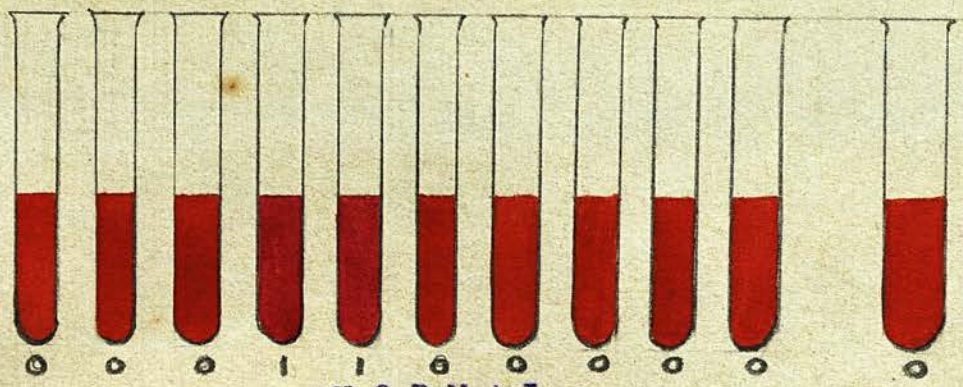




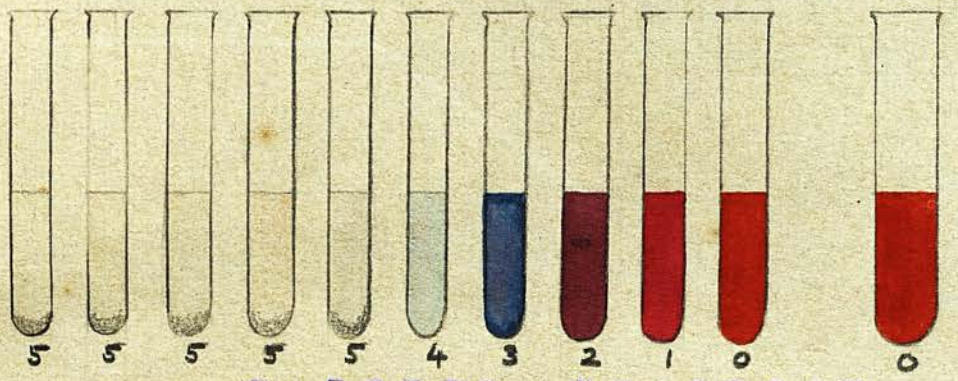




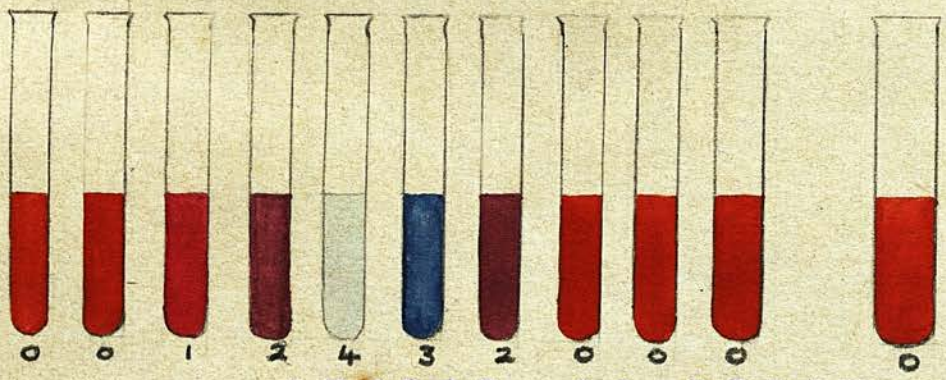
MENINGITIC REACTION



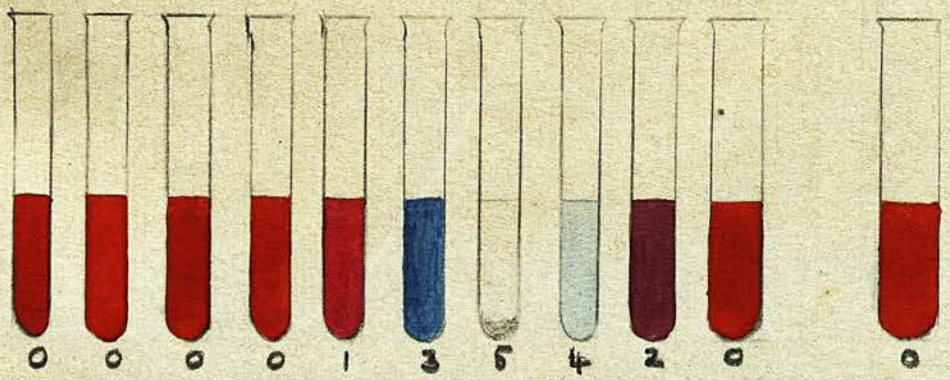
NORMAL



PARETIC REACTION



LUETIC REACTION



MENINGITIC REACTION

Note on the Mastic Test

A test similar in its purpose to the colloidal gold test has been introduced, in which the reagent is a colloidal solution of gum mastic: the technique of J.A.Cutting, as described by J.C.Todd,(4) was followed by the writer in a small series of cases. The precipitation of gold is here replaced by flocculation of the mastic solution from its suspension: the reagent is comparatively easily made and the method is thus free from certain drawbacks incidental to the gold (Lange) reaction: but even a limited experience of the method shows that it is much less sensitive than the gold reaction, because there are no longer the fine gradations perceptible when a colour reaction is available. It is impossible, e.g., to attempt a differentiation between dementia paralytica and cerebrospinal syphilis.

A representation of the reaction is given on the following page. (Plate II)

PLATE II

The Colloidal Mastic Test

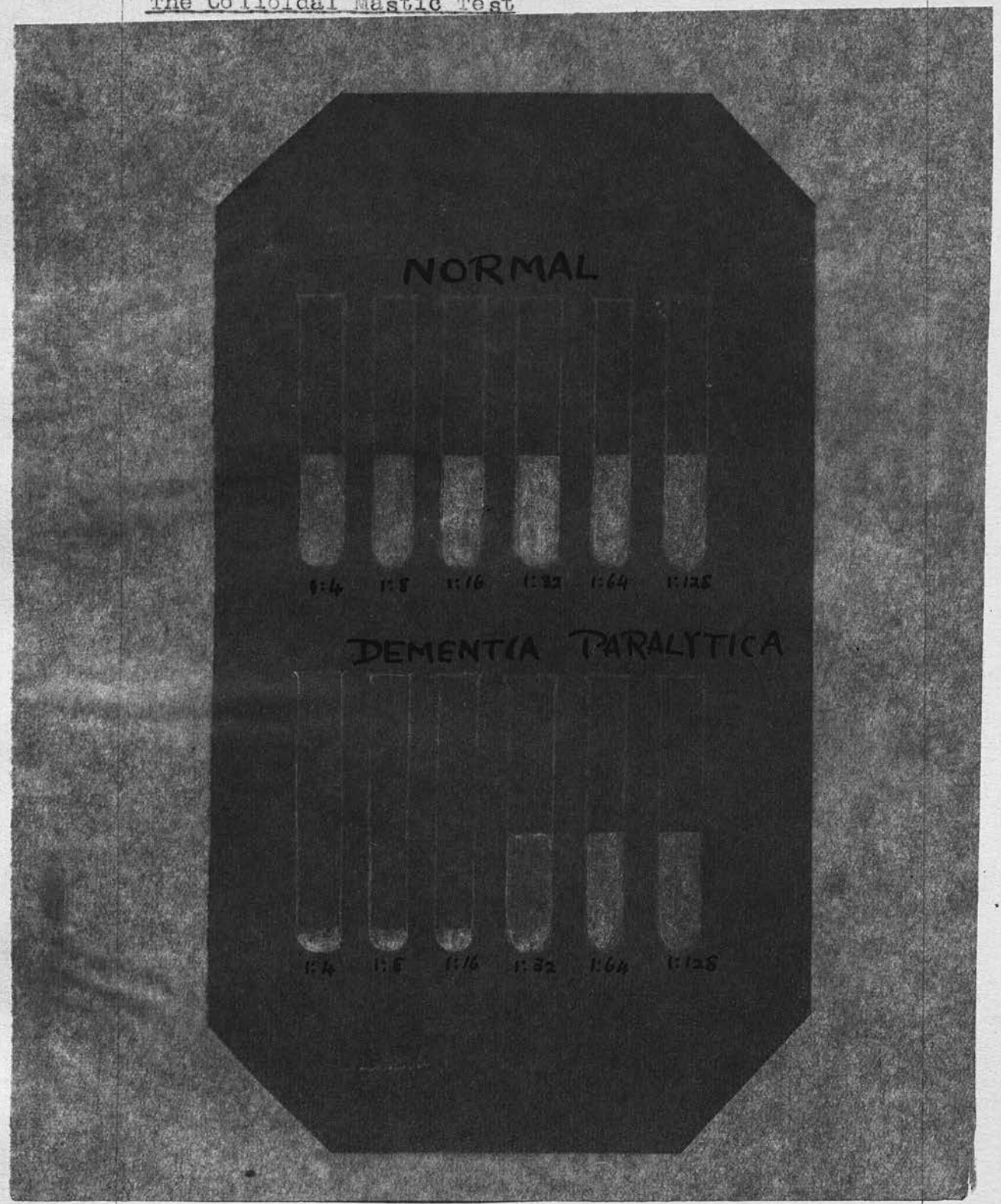
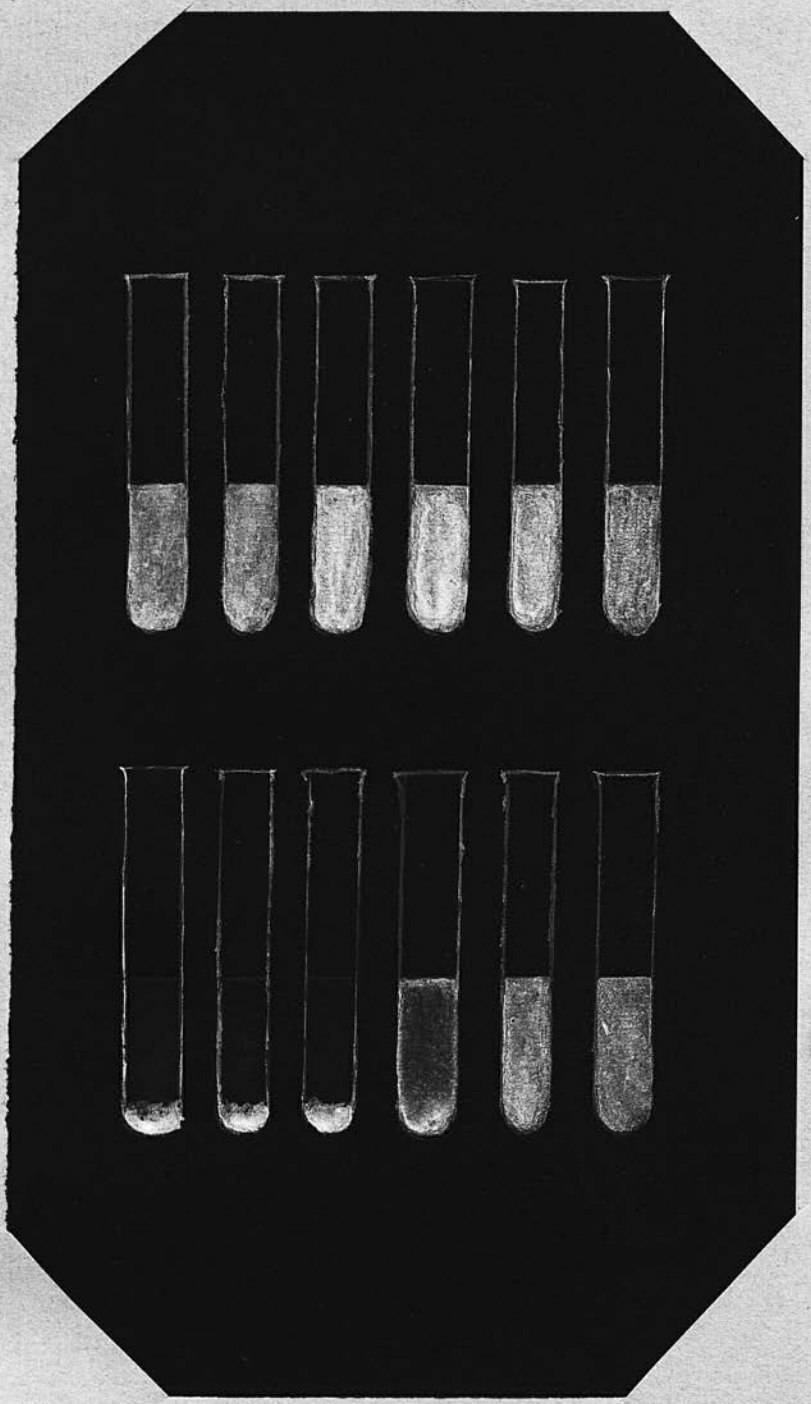


PLATE II

The Colloidal Mastic Test



(2) Analysis of cases examined

The present is a series of I23 cases: the laboratory notes regarding these have been tabulated in Appendix I. It has been found convenient to take them out of chronological order and arrange them in five tables, as follows:-

(1) Cases of dementia paralytica (general paralysis of the insane).

(2) Meningitis, other than syphilitic.

(3) Tabes & syphilitic meningitis.

(4) Various.

(5) Normal.

These headings may also be followed in discussing the results.

The tables also include the findings obtained when using certain other reactions, and the methods used in these investigations may be briefly noted here, for though not directly concerned in the colloidal gold reaction, it is of some importance to attempt to correlate the various observations.

The cells were invariably counted when the fluid was in a fresh state in a Thoma cell, and unstained: no method of combined staining and dilution was found satisfactory, and the examination of the fluid for cells in the"neat" state averts several sources of error.

Globulin excess was examined for by several methods, of which four were used chiefly, namely the

methods of Pándy,[24] Noguchi, Ross-Jones and Nonne-Appelt: the precipitants in these cases are respectively carbolic acid, butyric acid, ammonium sulphate as a ring test, and the same reagent added in equal quantity to the cerebrospinal fluid. In the writer's opinion, the method of Pándy using 1:15 carbolic acid is by far the simplest and most delicate, as well as requiring but one drop of the fluid being tested.

The Wassermann reaction in serum and cerebrospinal fluid was performed in the main after the manner described by Browning and Mackenzie:[5] latterly, however, the method used at Rochester Row by Harrison[6] has been followed. Some remarks are made later on the question of the amount of cerebrospinal fluid used for the test of complement-fixation.

(I) Cases of dementia paralytica.

It is at once seen from the first table in Appendix I, that the "paretic" reaction is remarkably constant: the feature is the complete precipitation of gold in the first four to eight tubes, and a gradual falling off of the reaction in the later tubes — the "step-ladder" curve. Kaplan[7] reports having found this in over 90%, and Craig[8] in 98-100% of cases. It may be noted that the reaction runs parallel with the Wassermann test, with the globulin reactions, and generally with a cell-count between 10 and 100 per c.mm. But the cell-count is often outside these limits. In cases 27 and 89, the blood Wassermann reaction was negative, while the cerebrospinal fluid Wasser-

mann, the gold test, and other findings, were positive. In no case were the Wassermann and gold test results discordant, but Miller et al[3] have reported cases with a positive gold and negative Wassermann reaction: the reverse, however, is unknown.

It is in this class of case that the most consistent and characteristic results are obtained.

If the cerebrospiral fluid is kept sterile and in the ice-chest, it remains capable of giving a typical reaction for a long time. Thus, the reaction of No. 5 was, on 9/9/'I6, 5555542I00: and on 27/3/'I7, about six months later, 5555554200, showing a difference which is negligible.

Fluid was taken repeatedly by lumbar puncture from the corpse in cases of dementia paralytica, and such fluid gave typical curves.

(2) Meningitis, other than syphilitic.

These cases show a curve which shows typically a maximum flocculation of gold about the junction of the middle and last third of the series of dilutions. On several occasions, it has been possible to examine the same specimen of fluid again at a later date: in each case, it was found that the reaction tends to gradually disappear. For example, in case No. II8, the reaction on I8/2/'I9 was 0000II3422

on 2I/2/'I9 0000023310

on I/3/'I9 0000012100

A collateral finding of interest for a later section of this thesis is the invariable presence of a large amount of protein, this being shown by heavy precipitates of globulin in the Pándy and other tests.

(3) Tabes: Cerebrospinal syphilis:

The cases of tabes noted in this table are cases in which the clinical diagnosis was apparent: judging by the reactions obtained, it is probable that a few cases placed in the category "various", should really find a place here: but these cases could not be traced clinically.*

It is stated by Miller and his co-workers(2) that the reaction in tabes is liable to contain more Nos. 4 than in other conditions, but they note that the reaction cannot be said to be typical. The few cases presented here of definite, clinical, tabes, cannot be said to possess any special characteristic.

One definite case of cerebrospinal syphilis is included.

(4) Various.

A case each of pernicious anaemia (101), senile dementia (14). and imbecility (13) gave a normal reaction.

In case 44, of Jacksonian epilepsy, the reaction 00I2300000 is slightly suggestive of a syphilitic meningeal process, but the Wassermann reaction was negative.

* In one case the spinal fluid W.R. was negative.

A case of pneumonia (22) giving a reaction of 000000II50 is interesting in view of the belief in some quarters that a meningitis of mild type is the rule rather than the exception in that disease: this case had no special nervous symptoms.

A case believed to be unique is No. 80, which gives the reaction 0I55555555. The fluid was xanthochromic, but did not show the "coagulation massive" described by Mestrezat.(13) Post-mortem, one found a number -- at least four -- of tumours of the cord, some of them confluent: these were found to have the structure of neurofibromata. Clinically, the case had been diagnosed as dementia paralytica.

(5) Normal

The great majority of these cases give an entirely negative reaction. But a number of fluids will be seen to give a reaction at first highly suggestive of a pathological condition, and the reason of this is the presence of blood. In order to assess the effect of the presence of blood in an otherwise normal fluid, some observations were made on the effect of adding blood to the fluid, normal and abnormal. In case No. 20, from a case of Dr. Graham Brown's, the reaction on II/12/'I6 was 00II23I000: this fluid contained blood to the extent of about 200 red corpuscles per c.mm. A further supply of fluid from the same patient obtained on 5/I/'I7 gave the reaction 000II00000: this fluid was free from blood. When a

little blood was added to this originally bloodless fluid, the reaction was 0000023410. Further, a dilution of haemoglobin made by adding such quantity of washed ox corpuscles as to give an intensity of colour similar to that of the fluid in the last experiment, gave the reaction 0001320000: the reason for this will appear in a later section. A small quantity of blood in the spinal fluid of a general paralytic will, in general, be insufficient to spoil the reaction, but it will tend to lessen it in the early tubes and extend it farther to the right of the series: on the other hand, the presence of blood in a normal fluid is liable, as Purves Stewart (9) remarks, to give misleading results: a few red corpuscles per c.mm. may, however, be ignored.

No. 26 was a normal fluid which had become septic: the reaction here, again, of 0000221100, is misleading. Another septic fluid (not included in the table) gave the reaction 2334433221. In order to test whether such a reaction is due to the presence of organisms as such, a suspension of staphylococci was made in distilled water: this failed to give any reaction with colloidal gold, however.

Comparison with other tests:

From the nature of the reaction it is evident that it must be more delicate than either the Wassermann reaction or the cell count: nor does the estimation of globulin by practicable methods approach a test which is given by such dilutions of cerebrospinal fluid as one in several hundreds or thousands. This tallies with several expressions of opinion, such as those of Kaplan,(7) Black and McBride,(10) Miller(2) and others.

Table (I) shows that the cell count in cases of dementia paralytica is usually under 100, as compared with the higher figures in acuter conditions: but exceptions are not uncommon, as cases 12, 40 and others show.

Apart from the special observations on the point to be recorded later, it is apparent that there is no necessary relation between the colloidal gold and the Wassermann reactions: although the series of cases shows that the two tests tend to run parallel, a case in Table (3) shows a positive gold reaction and a negative Wassermann reaction, the blood Wassermann reaction being positive. The conjunction of a positive Wassermann reaction and a negative gold reaction has never been encountered: nor has it occurred in Miller's experience.

Syphilitic cases apart, of course, one may get a gold reaction in the absence of any question of

syphilis or the Wassermann reaction, e.g., a case of tuberculous meningitis.

There appears to be no relation between the intensity of the Wassermann reaction and the type of colloidal gold curve: thus, case 12 required the use of 0.15 cc. of cerebrospinal fluid in order to fix 5 M.H.D. of complement, and the gold curve was 5555543200: whereas case 15 required only 0.05 cc. to fix the same amount of complement, yet the gold curve 5555553200 was similar or rather stronger. The observations were made on the same day under identical conditions.

(3) Researches into the nature of the reaction leading to a suggested explanation of some of the features.

Previous work on the subject:

Lange is inclined to think that there are different qualitative mixtures of protein substances which account for the reaction.

P.G.Weston (11) concluded from a study of the subject that the salts of the cerebrospinal fluid are incapable of causing the precipitation of gold which occurs: the copper-reducing substance could not be the cause: and he showed by dialysis and the ninhydrin reaction that amino-acids could not be considered the cause. He considers, however, that the gold-precipitating substance is dialysable and contrasts it in this respect with the Wassermann-reacting substance. These results will be further referred to.

Zsigmondy (17) had, in 1901 attempted to use colloidal gold solutions for the quantitative estimation of protein substances from a measurement of the degree of protection they conferred, and defined the "Goldzahl" of various proteins.

Zaloziecki (3) thinks that the reaction is an immunity phenomenon. Various objections to this theory will be brought forward.

Preliminary investigations:

On commencing an examination of the possible substances concerned in bringing about the reaction, it was possible to exclude some of these readily.

The cells of the cerebrospinal fluid cannot be responsible, since a filtrate or the supernatant fluid after centrifuging is equally competent.

The reaction cannot be simply a flocculation of colloidal gold from the electrolytic action of the salts of the spinal fluid, for the concentration of such salts never reaches that which would be capable of acting in this way: the amount of mineral matter in the cerebrospinal fluid is about 0.87% (Mestrezat)[13], chiefly as chloride and bicarbonate, and this amount is not appreciably deviated from in pathological conditions: even in the first dilution of cerebrospinal fluid with 0.4% saline, the concentration of electrolyte would be about 0.6% and that would be quite incapable of affecting the gold solution.

As to the copper-reducing substance, the mere fact that it is absent in cerebrospinal meningitis (meningococcal) while that fluid gives a gold reaction, rules it out.

Cholesterin (and cholesterin esters) is only to be found in the cerebrospinal fluid of normal individuals in traces (Mestrezat[13], Mott[14])*. On the supposition that, since the reaction is given by the cerebrospinal fluid from individuals whose condition is such that there is presumably a wasting of nerve tissue, there might be a substance of a lipoid nature responsible for the reaction, an attempt was made to elucidate

* Also Weston.[12]

this point. In one type of experiment there was mixed with a normal cerebrospinal fluid an emulsion of lecithin: the mixture was then placed at 37 deg. for some hours in order to allow any combination with protein or other substance to take place: the fluid was then tested with colloidal gold in various dilutions, but without any result: the same failure occurred when using cholesterin in a similar way. Other experiments* were directed towards the estimation of cholesterin in normal and abnormal spinal fluids, using the method of Grigaut (as described by Plimmer)(15), but the amount was inestimable: Bloor's(16) method was also tried but with no better success.

It was accidentally found that a solution of Witte's "peptone" had in a powerful degree the faculty of precipitating colloidal gold: but the facts that it is readily dialysed through collodion sacs and that the reaction with "peptone" persists after the solution has been boiled, demonstrate that this substance -- a mixture of substances -- cannot be at work in the colloidal gold reaction: indeed, the fact that there is no protection of gold solution in any dilution is highly suggestive that the precipitation that occurs is really of the type caused by an electrolyte.

Certain considerations made it highly probable that the colloidal gold reaction is in some way due to, or associated with the protein of the cerebrospinal fluid: there is the invariable association of a positive reaction with an increased globulin con-

* In these experiments a Duboscq colorimeter was used which was kindly lent by the Medical Research Committee.

tent: and boiling of a cerebrospinal fluid from a case of dementia paralytica, even for a short time, was found to destroy its power of giving the reaction. All the subsequent observations have gone to show that protein is the substance responsible for the reaction. But the problem is more complicated than it would be if it were simply a matter of protein precipitating gold under any circumstances: for a fluid from a case of cerebrospinal meningococcal meningitis, although extremely rich in protein does not precipitate gold from its colloidal solution when in a dilution of 1:10, whereas a spinal fluid from a general paralytic will do so, in spite of the fact that its protein content is very much lower.

Relation of protein to the gold reaction.

Some phenomena of colloidal chemistry may be mentioned here inasmuch as they have guided the researches recorded in this section. The authorities consulted have been, chiefly, Bayliss,(17) Burton(18) and Wells.(19) The colloidal solution of gold used in the test is one of the most sensitive of such metallic suspensoids: the sensitivity is measured by the coagulating (precipitating) effect of electrolytes added to the solution: when, for example, 1.7 cc. of 1% NaCl are added to 5 cc. of colloidal gold solution, there occurs within an hour a series of colour changes from the original red-orange through purples and ~~reds~~ blues to complete decolorisation, the gold being precipitated to the bottom of the tube. A colloidal gold solution which does not behave in this fashion is described as

"protected" and is of no use for the test. The particles of colloidal gold carry an electric charge, which gives the solution the properties of an electronegative suspenoid. (This applies to the red gold solutions, -- such as are used in the test: it was shown by Morris-Airey and Lang[20] that in blue solutions, the reverse is the case, the particles being positively charged.)

Now the other colloid concerned in the problem is the protein of the cerebrospinal fluid -- chiefly globulin: in the body fluids, protein is an emulsoid, and unlike the metallic suspensoids it is comparatively highly resistant to the coagulative action of electrolytes. It was shown by Hardy[21] that the electrical charge on particles of globulin is determined by their chemical reaction: this is due to the amphoteric nature of the protein molecule as a complex of amino-acids, since they carry both NH_2 groups and COOH groups. Proteins may, in fact, behave either as weak acids or weak bases and the electrical charge is accordingly variable.

When two colloids of opposite sign are mixed, there occurs mutual precipitation provided that the proportions are optimal for that reaction: on the other hand, should either be in excess, the protective action referred to comes into play and there is no visible change.

Hypothesis suggested:

From these data it became possible to construct a hypothesis as to the mechanism of the colloidal gold reaction. In the writer's belief, the reaction which takes place is determined by the quantitative relations of two colloids of opposite electrical sign -- the electronagative gold solution and the electropositive protein solution.

As already mentioned, the sign of electrical charge on protein particles in solution is decided by the reaction: in the case of cerebrospinal fluid, the reaction is alkaline: the globulin may be regarded as forming a salt with the base present -- say, a sodium globulinate: such a salt is electrolytically dissociated in solution, into sodium and an organic ion which has the properties of the colloidal state. As a weak base, the protein gives off OH ions and is left, itself, positively charged.

We have thus the electropositive protein and the electronegative gold particles -- assuming always that the protein solution is alkaline, as it is in the normal and pathological spinal fluid. If these two colloids be mixed in suitable proportions, there will result mutual precipitation: it is the gold which appears to form the precipitate, but in reality the precipitated or flocculated particles consist of both the colloids. It is believed (Burton)[18] that what happens is that at the time of maximum precipitating action, the numbers of each kind of particle -- negative and positive -- produce uncharged masses which coal-

esce and fall to the bottom of the tube. The question of precipitation of the gold by the electrolyte present as salts may be ruled out of consideration, for the concentration of electrolyte present would be quite insufficient to have any action: in fact, the solution used in making the dilutions of cerebrospinal fluid for the test is 0.4% NaCl, a strength chosen because, while sufficient to keep the protein in solution, it is not sufficient to have any precipitating action on the colloidal gold.

When, however, the relative quantities of the two colloids are not those conducive to mutual precipitation, there occurs protection from the coagulative action of electrolytes when they are added subsequently to the mixture: it is believed that the particles of colloid in excess -- in the present case protein -- form a film round the gold particles: the complex then takes on the characters of protein -- an emulsoid -- and is highly resistant to the precipitating action of added electrolytes: the addition to 5 cc. of such a protected gold solution of I.7 cc. of 1% NaCl (and indeed much larger amounts, according to the degree of protection) will be without effect.

A number of experiments have been carried out to test this hypothesis, and these have proved to be confirmatory. If the reaction be due to globulin, it ought to be possible on isolating this substance from the cerebrospinal fluid to reproduce the reaction with it: this has been done: a quantity of cerebrospinal fluid from several cases of dementia paralytica was

half saturated with Ammonium sulphate, and the precipitate -- representing the globulin fraction -- collected and placed in a Soxhlet extraction thimble impregnated with collodion by a 10% solution of that substance in alcohol and ether: the globulin was then dialysed against distilled water frequently changed for several days: precautions were taken against bacterial contamination: in this way the salt was removed until the dialysate showed no reaction for ammonium. It is true that the salt cannot be entirely removed from the globulin, but it can be reduced below that amount which would have any effect on the gold solution by salt action. When globulin prepared in this way is mixed in a series of dilutions with colloidal gold, there occurs a precipitation in certain dilutions, but this cannot be compared with the reaction previously obtained with the "neat" fluid: it was then observed that the factor of reaction had been neglected: when the reaction was made slightly alkaline (alizarin red), the globulin solution precipitates gold very readily in certain proportions and protects it in others. It is possible to imitate the various types of reaction by varying the concentration of the globulin solution.

Nor is the reaction peculiar to the globulin of the cerebrospinal fluid: the globulin obtainable from serum behaves in a similar manner.

Before this was established, it was thought desirable to investigate the relation, if any existing between the gold reaction and the Wassermann reaction. To this end two kinds of experiments were carried out.

It is known that the Wassermann reaction is closely concerned with the globulin moiety of the serum protein: that the cerebrospinal fluid in dementia paralytica gives a positive reaction: that there is an excess of globulin in such a cerebrospinal fluid: and that such a fluid gives a colloidal gold reaction. The question arose as to how far these findings can be correlated.

One set of experiments*, therefore, was carried out with globulin prepared from blood serum --(1) normal, and (2) Wassermann-reacting. These preparations were tested for the Wassermann reaction, and for the gold reaction: the technique followed in preparing the serum globulin was the same as that detailed for the spinal fluid globulin (supra). It was found that the globulin fraction from Wassermann-reacting serum gave a positive reaction, while that from normal serum failed to deviate complement. This is in confirmation of such researches as those of Noguchi, Landsteiner & Müller, and others (quoted by Browning and Mackenzie(5)). On the other hand, these specimens of globulin, though differing in their behaviour in the complement-fixation test, act in an identical manner when put up in a variety of dilutions with colloidal gold. It follows that the attribute of syphilitic globulin which gives origin to a positive Wassermann test has no relation to that causing the precipitation of gold in the colloidal gold test. So far as the gold reaction is concerned, the globulin of syphilitic serum does not differ from that of normal serum.

* Protocols placed in Appendix II (page 55)

A second series of experiments* was directed towards establishing the identity or otherwise of the serum globulin with that of cerebrospinal fluid: this was attempted by means of the precipitin reaction, and this was incidentally used in investigating the same subject as is dealt with by the last paragraph. A number of rabbits were injected with serum, some with normal and others with Wassermann-reacting serum: the first injection was in all cases into an ear vein, and the remainder intraperitoneal, the technique described by G.H.F.Nuttall (22) being followed in essentials: after a number of these injections, the animals were bled and the serum of each collected: on testing these sera with the homologous sera, precipita were obtained: on testing a Wassermann-serum-antiserum against a normal serum, the same amount of precipitum was obtained: and when the cerebrospinal fluid from a case of dementia paralytica was tested, it was immaterial so far as the amount of precipitum was concerned, whether a normal-serum-antiserum, or a Wassermann-serum-antiserum were used. Thus, so far as the precipitin reaction is informative, there is no difference between the proteins of normal & Wassermann reacting sera and cerebrospinal fluid (Wassermann-reacting).

It may be concluded that the globulin in general paralytic spinal fluid is identical -- so far as these investigations can tell -- with serum-globulin, whether normal or Wassermann-reacting. It thus becomes very unlikely that the gold reaction bears any relation to an immunity phenomenon: certainly it bears no necessary relation to the Wassermann reaction. This

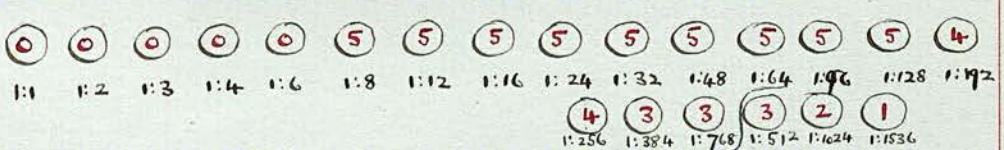
* Protocols in Appendix II (page 55)

had already appeared unlikely from some observations recorded in the previous section. It is accordingly inaccurate, at least, to say that "Lange's gold test is believed to be specific for syphilis."(23) And this is quite apart from the fact that a gold reaction -- albeit of a different type -- is obtainable in a variety of conditions where no question of syphilis is involved.

(It has since been found that Mestrezat(13) and Fleig, using the precipitin reaction, had already come to a conclusion similar to that above stated with regard to the identity of spinal fluid globulin with serum globulin.)

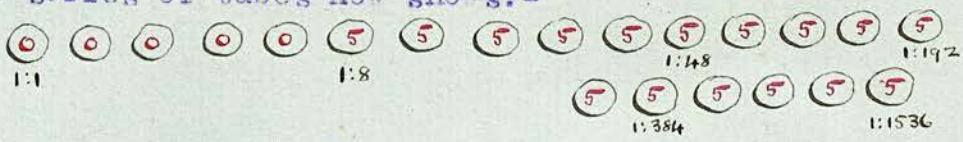
It is possible now to return to the hypothesis put forward and offer explanation of some other features of the reaction.

It would appear at first sight that in the case of a cerebrospinal fluid from a case of dementia paralytica, giving a reaction of 5555543210, the protein was precipitating gold in all dilutions higher than 1:160 (the dilution in the fifth tube): but this is not so: if, in addition to the usual dilutions put up in the test, some more concentrated dilutions are made, it is found that the precipitation of gold does not commence until the dilution reaches about 1:8, so that a more extended curve is as follows:-



And when protection is tested for by adding 1.7 cc. of 1% NaCl to the tubes, it is found that the tubes ante-

cedent to that with the dilution 1:8 are protected. The series of tubes now shows:-



Thus the globulin does protect in some dilutions while it precipitates in others.

Now, in a case of meningococcal meningitis, the type of reaction is 0000123420: this is to be associated with the fact that such a fluid contains a relatively large amount of protein, so much in fact as to protect the gold solution up to a dilution much higher, about 1:200, as compared with the 1:8 in dementia paralytica.

A refernce to the analyses given by Mestrezat (13) amplifies this conclusion: he does not deal with the gold reaction, but has estimated the protein content of various cerebrospinal fluids by direct chemical methods: some average figures are as follows:-

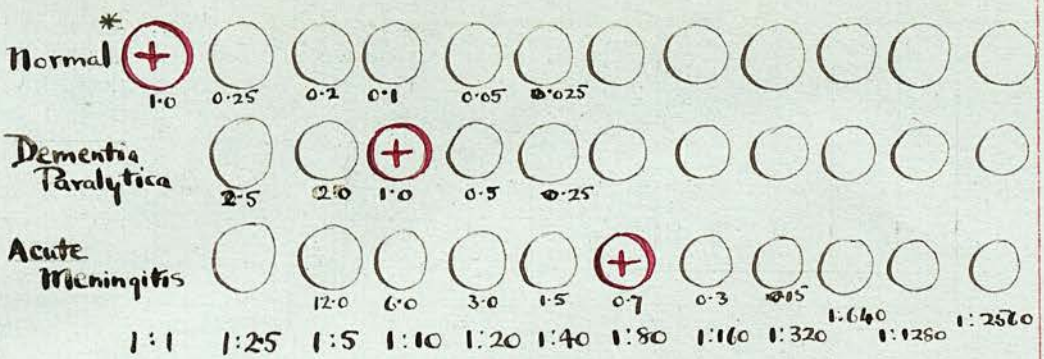
Normal - - - - - - 0.18 gm. per litre

General paralysis -1.00 " " "

Cerebrospinal & Tuberculous meningitis. -6.00 " " " (and upwards)

H.M.Adler (25) has also shown the increase of protein by estimations of the total nitrogen.

The ratios are thus about 1:10:60: and if a table be constructed to show a series of dilutions of each of these, it is found that the point of first precipitating effect coincides with that dilution which gives the same amount of protein in each, thus:-



It is believed, then, that the various types of reaction are to be ascribed to the varying quantitative relations of globulin in the various fluids.

The curves resulting have also a clinical correlation: an acute disease such as meningococcal meningitis is accompanied by a large amount of inflammatory exudation -- and the protein content is accordingly high: in dementia paralytica, on the other hand, the process is comparatively chronic and is less affecting the meningeal structures which provide exudate than the nerve tissue itself. A subacute condition such as syphilitic meningitis represents an intermediate between these two and gives the intermediate type of reaction -- precipitation commencing at a greater degree of dilution than in dementia paralytica, but at a lower dilution than in acute meningitis. The single case in the series recorded giving the unusual reaction of OI55555555 had a very large amount of protein in the cerebrospinal fluid, such that even a dilution of 1:5120 was capable of precipitating the gold.

The amount of globulin is to be further

* A normal fluid in the dilution 1:1, induces some precipitation of gold.

correlated with the number of cells in the fluid -- low in dementia paralytica (usually under 100), higher in a subacute meningitis e.g. syphilitic, and amounting to pus in acute meningitis.

Observations made with the albumin fraction obtained as the filtrate after half-saturation of cerebrospinal fluid or serum seemed to show that the protective effect of that protein is sustained through a longer series of dilutions: owing to the minute quantity present in the spinal fluid, its effect is probably overshadowed by the other protein -- globulin -- present.

C O N C L U S I O N S

Arising from Section (I)

The colloidal gold reaction is one of simple technique after a satisfactory purity of distilled water has been attained: this should not hinder its adoption as a test, for the gold solution can be prepared by a central laboratory and supplied from there. Apart from the preparation of the solution, there is no specially difficult phase in the procedure.

Arising from Section (2)

From an examination of over 123 spinal fluids it is concluded that the test is one of considerable value as a diagnostic procedure.

The results in dementia paralytica are so constant as to justify the use of the term "paretic reaction": but like other tests, this one has limitations: it is an adjunct of great service in the laboratory diagnosis of nervous affections, but not in itself a crucial test for any specific condition.

Since the nagative Wassermann reaction and positive bacteriological findings will generally exclude disease other than syphilitic, one of the chief uses of the test is likely to be in the differential diagnosis of syphilitic nervous affections: for the Wassermann reaction, though positive in a great majority of cases, fails to give information as to the type of disease present: and the globulin and cytolog-

ical results are likewise apt to be equivocal. The colloidal gold test here fills a gap in clinical pathology.

The information given by the test is all the more valuable in view of the great extent to which an accurate diagnosis of syphilitic nervous disease controls the prognosis of the case. It is by the use of such a reaction as this that the earliest stages — still, perhaps amenable to treatment -- of general paralysis are likely to be detected.

Arising from Section (3)

The reaction has a considerable scientific interest.

Various experimental observations have been adduced in support of the hypothesis that the essential cause of the reaction is the globulin of the cerebrospinal fluid

It is believed that the various types of reaction are explicable by the varying amounts of globulin present in the spinal fluid in various conditions.

The mechanism of the reaction consists in the mutual precipitation which occurs when two colloids of opposite electrical sign are brought into contact.

The importance of the alkaline reaction of the cerebrospinal fluid for this reaction has been explained.

Various considerations have shown the absence of any necessary relation between the colloidal gold and the Wassermann reactions. Syphilitic globulin, as opposed to normal globulin, gives a positive Wassermann reaction: but so far as the precipitin and gold tests are informative on the point these globulins are identical, nor do they differ from the globulin which occurs in the cerebrospinal fluid.

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APPENDIX I

Results of examination of cerebrospinal fluids, arranged in tables:-

(1) - - - Cases of dementia paralytica (G.P.I.)

(2) - - - Cases of meningitis, other than syphilitic.

(3) - - - Cases of tabes and syphilitic meningitis.

(4) - - - Various.

(5) - - - Normal.

Reference from Numbers to Tables

Table (1)	Table (2)	Table (3)	Table (4)	Table (5)
				1-2
3				
				4
5-9				
				10
11-12				
			13-14	
				15-19
20-21				
			22	
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				24-26
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30-31				
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62-63				
				64-68
69-71				
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73-76		77		
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	82			
83-84				85-86
87-90				
		91		
				92-94
95-98				
				99
100				
			101	102-106
107				108
		109		
110		111		
			112	113
		114-5		
	118			116-7
120				119
				121-2
		123		

Table (I) — Dementia Paralytica.

No.	Date	Cells per c.mm.	Globulin Tests: Pandy	Noguchi	Ross-Jones	Nonne-Appelt	Wassermann Reaction: Seram	C.S.F.	Colloidal Gold Test: 1	2	3	4	5	6	7	8	9	10	Clinical, Etc Notes
3	30/8/16	130 [R.B.C. 300]	+	+	+	+	+	+	5	5	5	5	5	3	2	1	0	0	Paresis – G.P.I. Repeat on 9.11.'16 gave same reaction
5	9/9/16	21	+	+	+	+	+	+	5	5	5	5	5	4	2	1	0	0	Paresis. – G.P.I.
6	10/9/16	97	+	+	+	+	+	+	5	5	5	5	4	3	1	0	0	0	G.P.I. Slight blood contamination.
7	20/9/16	118	+	+	+	+	+	+	5	5	5	3	3	3	0	0	0	0	G.P.I Death after cerebral seizure: G.P. verified P.M.
8	27/9/16	150	+	+	+	+	+	+	5	5	5	5	5	2	2	0	0	0	G.P.I. (Some blood contamination)
9	2/10/16	26	+	+	+	+	+	+	5	5	5	5	5	5	3	1	0	0	G.P.I. Verified P.M.
11	9/10/16	Fluid heavily contaminated with blood	(+	+	+	+)	+	+	5	5	5	5	5	5	5	3	1	0	G.P.I. = Repeat on 15.12.'16, when free of blood.
									5	5	5	5	4	3	2	1	0	0	
12	28/11/16	125	++	+	+	+	+	+	5	5	5	5	5	4	3	2	0	0	G.P.I. of the classical type.
15	28/11/16	15 [R.B.C. 77]	++	+	+	+	+	+	5	5	5	5	5	5	3	2	0	0	G.P.I. not of classical type
16	28/11/16	20	++	+	+	+	+	+	5	5	5	5	5	5	5	3	1	0	G.P.I. also with tabes

Table (I) — continued.

No.	Date	Cells per c.mm.	Globulin Tests: Pandy	Noguchi	Ross-Jones	Nonne-Appelt	Wassermann Reaction: Serum	C.-S.F.	Colloidal Gold Reaction: 1	2	3	4	5	6	7	8	9	10	Clinical, etc Notes
17	28/11/16	7 [R.B.c. 70]	++	+	+	+	+	+	5	5	5	5	5	5	3	1	0	0	Early G.P.I.
18	28/11/16	8 [R.B.c. 74]	+	+	+	+	+	+ weak	5	5	5	5	5	5	1	1	1	0	Acute G.P.I.
19	28/11/16	12	++	+	+	+	+	+	5	5	5	5	5	5	3	2	0	0	Early G.P.I.
23	3/1/17							+	5	5	5	5	4	2	1	0	0	0	G.P.I. (per Dr. Graham Brown)
27	10/1/17	7	+	Faint +	Faint +	Faint +	—	+	5	5	5	5	4	2	0	0	0	0	G.P.I., florid stage
30	16/1/17	76	++	+	+	+	+	+	5	5	5	5	5	5	4	2	1	0	G.P.I.
31	16/2/17	31 [R.B.c. 3]	++	+	+	+	+	+	5	5	5	5	5	4	3	1	0	0	G.P.I. Pin-point pupils.
33	20/2/17								5	5	5	5	4	3	1	0	0	0	G.P.I. (per Dr. Graham Brown)
34	20/2/17	20	++	+	+	+	+	+	5	5	5	5	5	5	3	2	1	0	G.P.I.
38	20/3/17	25	+	+	+	+	+	+	5	5	5	5	5	5	4	3	2	1	G.P.I.

No.	Date	Cells per c.mm.	Globulin Tests: Pandy	Noguchi	Ross-Jones	Nonne-Appelt	Wassermann Reaction: Serum	C.S.F.	Colloidal Gold Reaction: 1	2	3	4	5	6	7	8	9	10	Clinical, etc. Notes
39	20/3/'17	98	+	+	+	+		+	5	5	5	5	5	5	4	3	2	1	G. P. I.
40	20/3/'17	120	+	+	+	+	+	+	5	5	5	5	5	5	5	4	3	2	G. P. I.
41	20/3/'17	20	+	+	+	+	+	+	5	5	5	5	5	5	3	2	1	0	G. P. I. Convulsions.
42	20/3/'17	16	+	+	+	+		+	5	5	5	5	5	5	4	3	2	1	G. P. I.
43	20/3/'17	10	+	+	+	+	+	+	5	5	5	5	5	3	3	2	1	0	G. P. I.
49	29/3/'17	20	+	+	+	+	+	+	5	5	5	5	5	5	4	3	2	1	G. P. I.
50	29/3/'17	16	+	+	+	+	+	+	5	5	5	5	5	5	4	3	2	1	Early G. P. I.
51	29/3/'17	37 [R.B.C. 1260]	++	+	+	+	+	+	5	5	5	5	5	5	3	2	1	0	G. P. I. Curve exaggerated by presence of blood.
52	2/4/'17	Fluid drawn p.m. — contaminated.					+	+	0	0	0	1	4	5	3	4	2	2	G. P. I. — p.m. Atypical curve due to excessive serum.
54	6/4/'17	35	+	+	+	+	+	+	5	5	5	5	5	5	5	4	2	1	G. P. I.

Table (1) — continued

No.	Date	Cells per c.mm.	GLOBULIN TESTS Pandy	Noguchi	Nonne-Appelt	Ross-Jones	WASSERMANN Reaction Serum	C.-S.F.	Colloidal Gold Test 1 2 3 4 5 6 7 8 9 10	Clinical, Etc. Notes
62	7/5/'17	27	+	+	+	+	+	+	5 5 5 4 2 1 0 0 0 0	G. P. I. } Same case on different dates.
63	28/5/'17	30	+	+	+	+	+	+	5 5 5 3 2 1 0 0 0 0	G. P. I. } Same case on different dates.
69	29/5/'17	30	+	+	+	+	+	+	5 5 5 5 5 2 1 0 0 0	G. P. I.
70	30/5/'17	103	+	+	+	+	+	+	5 5 5 5 5 3 2 1 0 0	G. P. I. Of 333 cells counted:- Lymphocytes 53 %, Polymorphs 41 %, Eosinophils 6 %
71	10/6/'17	26	+	+	+	+	+	+	5 5 5 5 4 2 0 0 0 0	G. P. I.
73	10/6/'17	40	+	+	+	+	+	+	5 5 5 5 2 2 0 0 0 0	G. P. I.
74	10/6/'17	18	+	+	+	+	+	+	5 5 5 5 5 4 3 2 0 0	G. P. I.
75	19/6/'17	56	+	+	+	+	+	+	5 5 5 4 4 3 2 1 0 0	G. P. I.
76	27/6/'17	32	+	+	+	+	+	+	5 5 5 5 4 1 1 0 0 0	G. P. I.
78	27/6/'17	136	+	+	+	+	+	+	5 5 5 5 4 4 4 0 0 0	G. P. I.

Table (II) — continued

No.	Date	Cells per c.mm.	Globulin Tests: Pándy	Noguchi	Ross-Jones	Nonne-Appelt	Wassermann Reaction: Serum	C.-S.F.	Colloidal Gold Reaction: 1	2	3	4	5	6	7	8	9	10	Clinical etc. Notes
81	21/7/17	Lumbar Puncture p.m.	++	++	++	+	+	+	5	5	5	5	5	4	3	2	0	0	G. P. I. verified p.m. Excessive protein
83	6/8/17	14	+	+	+	+	+	+	5	5	5	5	5	4	1	1	1	1	G. P. I.
84	6/8/17	50	+	+	+	+	+	+	5	5	5	5	5	5	3	1	0	0	G. P. I.
87	6/8/17	52	+	+	+	+	+	+	5	5	5	5	5	5	5	4	3	0	G. P. I.
88	6/8/17	42	+	+	+	+	+	+	5	5	5	5	5	4	3	0	0	0	G. P. I.
89	18/8/17	38	+	+	+	+	−	+	5	5	5	5	5	4	4	3	0	0	G. P. I. (Crookston War Hosp.)
90	23/8/17	26	+	+	+	+	+	+	5	5	5	5	5	4	1	1	0	0	G. P. I.
95	23/8/17	24 [R.B.C. 49]	+	+	+	+		+	5	5	5	5	5	5	3	0	0	0	G. P. I.
96	24/8/17	36 [R.B.C. 24]	+	+	+	+	+	+	5	5	5	5	4	3	1	0	0	0	G. P. I.
97	24/8/17	20	+	+	+	+		+	5	5	5	5	5	5	4	3	0	0	G. P. I.

Table (I) — continued

No.	Date	Cells per c.mm.	Globulin Tests: Pandy	Noguchi	Ross-Jones	Nonne-Appelt	Wassermann Reaction: Serum	C.S.F	Colloidal Gold Reaction: 1	2	3	4	5	6	7	8	9	10	Clinical, etc Notes
98	24/8/17	48	+	+	+	+	+	+	5	5	5	5	5	5	4	4	1	0	G. P. I.
100	30/8/17	25	+	+	+	+		+	5	5	5	5	5	5	3	2	1	0	G. P. I. (Crookston War Hosp.)
107	10/9/17	8	+	+	+	+	+	+	5	5	5	5	3	1	0	0	0	0	G. P. I.
110	10/9/17	Heavy Contamination with blood	(+	+	+	+)	+	+	5	5	5	5	4	3	2	1	0	0	G. P. I., but interfered with by blood.
120	5/3/19	18	+					+	5	5	5	5	4	3	1	0	0	0	G. P. I. — early (Brighton V.D. Clinic)

Table (I) — concluded

Table (8) — Meningitis, other than syphilitic

No.	Date	Cells per c.mm.	Globulin Tests: Pandy	Noguchi	Ross-Jones	Nonne-Appelt	Wassermann Reaction Serum	C.-S.F.	Colloidal Gold Reaction: 1	2	3	4	5	6	7	8	9	10	Clinical, etc. Notes
35	4/3/'17		++	++	++	+			0	0	0	1	2	3	1	0	0	0	Tuberculous meningitis. (per Miss Fitzgerald, R.D.E.)
46	27/3/'17							—	0	0	0	0	1	2	3	2	1	0	Tuberculous meningitis (per Miss Fitzgerald, R.D.E.)
61	5/5/'17	Purulent — polymorph.	+++	++	++	++			0	0	0	0	0	1	4	3	2	2	Cerebrospinal meningitis — meningococcal. First puncture. Recovery.
82	21/7/'17		++	+	+	+		—	0	0	0	0	1	3	2	1	0	0	Tuberculous meningitis: (per Miss Fitzgerald, R.D.E.)
118	18/2/'19	very purulent	+++	+++	+++	+++			0	0	0	0	1	1	3	4	2	2	Meningococcal meningitis
118a	21/2/'19								0	0	0	0	0	2	3	3	1	0	Same specimen as above: to show fading of the reaction.
118b	1/3/'19								0	0	0	0	0	1	2	1	0	0	Ditto, at still later date.

No.	Date	Cells per c.mm.	Globulin Tests: Tandy	Noguchi	Ross-Jones	Nonne-Appelt	Wassermann Reaction: Serum	C.S.F.	Colloidal Gold Reaction: 1	2	3	4	5	6	7	8	9	10	Clinical, etc. Notes
47	27/3/'17		+	+	+	+		+	5	5	5	5	4	4	2	1	0	0	Clinically tabes, but doubtful: not traced. (per Dr. Graham Brown)
77	27/6/'17	28	+	+	+	+	+	−	1	2	2	3	3	4	1	0	0	0	? Tabes
109	10/9/'17	12	+	+	+	+	+	+	0	0	5	5	4	3	2	2	0	0	? Tabes or tabo-paresis
111	15/9/'17	0	+					+ weak	0	0	1	1	4	3	3	3	1	0	Classical tabes (Cumberland War Hosp.)
91	23/8/'17	42	+	+	+	+	+	+	1	1	1	2	5	4	3	1	0	0	Same case as 77
114	4/2/'19	200	++	+	+	+	+	+	0	0	1	4	3	2	0	0	0	0	Syphilitic meningitis. (Brighton V.D. Clinic)
115	17/2/'19	19	+				+	+	3	5	5	5	4	4	2	1	0	0	Tabes: clinically, incontinence of urine & faeces (Royal Sussex County Hosp.)
115a	4/3/'19	42	+				+	+	4	4	5	4	3	2	2	0	0	0	Same case at later date.
123	5/3/'19	23	+				+	+	0	0	1	4	3	3	2	1	0	0	Tabes (Royal Sussex County Hosp.)

No.	Date	Cells per c.mm.	Globulin Tests: Pandy	Noguchi	Ross-Janes	Nonne-Appelt	Wassermann Reaction: Serum	C.-S.F.	Colloidal Gold Reaction: 1	2	3	4	5	6	7	8	9	10	Clinical, etc. Notes
13	28/11/16	0	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	Imbecile
14	28/11/16	12	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	Senile Dementia: Excited & arteriosclerotic
22	16/12/16	73	+	?	?	?	—	—	0	0	0	0	0	0	1	1	5	0	Pneumonia: no cerebral symptoms.
44	27/3/17							—	0	0	1	2	3	0	0	0	0	0	Jacksonian Epilepsy ?Syphilitic
45	27/3/17							—	0	0	1	2	3	3	2	1	1	0	? Not diagnosed: Not traced clinically (Sick Childrens' Hosp., Edin.)
80	27/6/17	48 Xanthochromia	++	++	++	++	—	—	0	1	5	5	5	5	5	5	5	5	Clinically, G.P.I. P.M., Neurofibromata of Cord
101	2/9/17	9	—	—	—	—	—	—	0	0	1	1	0	0	0	0	0	0	Pernicious anaemia. Lumbar punct. p.m.
112	2/9/17	Heavy Contamination with Blood					+	—	1	1	1	2	5	4	3	1	0	0	Not traceable clinically: ? partly due to blood contamination.

No.	Date	Cells per c.mm.	Globulin Tests: Pándy	Noguchi	Ross-Jones	Nonne-Appelt	Wassermann Reaction: Serum	C.S.F.	Colloidal Gold Reaction: 1	2	3	4	5	6	7	8	9	10	Clinical, etc. Notes
1	26/8/16	8	—	—	—	—	—	—	0	0	0	0	0	1	0	0	0	0	Pseudo-tabes
2	26/8/16	4	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	Normal
4	10/9/16	1	—	—	—	—	—	—	0	0	1	1	0	0	0	0	0	0	Normal
10	17/11/16	0	—	—	—	—	—	—	0	0	0	1	1	0	0	0	0	0	Normal
20	11/12/16	Much contamination with blood						—	0	0	1	1	2	3	1	0	0	0	? Cerebrospinal syphilis. But reaction due to blood. (per Dr. Graham Brown)
21	11/12/16							—	0	0	1	1	0	0	0	0	0	0	? Cerebrospinal syphilis. (per Dr. Graham Brown)
24	3/1/17							—	0	0	0	1	1	0	0	0	0	0	Ditto.
25	3/1/17							—	0	0	0	1	1	1	1	1	0	0	Ditto.
26	3/1/17	Fluid Septic							0	0	0	0	2	2	1	1	0	0	Reaction due to Septic fluid (per Dr. Graham Brown)
28	10/1/17	7	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	Mental — obscure.

Table (5) — Normal

Table (5) — continued

No.	Date	Cells per c.mm.	Globulin Tests: Pandy	Noguchi	Ross-Jones	Nonne-Appelt	Wassermann Reaction: Serum	C.S.F.	Colloidal Gold Reaction: 1	2	3	4	5	6	7	8	9	10	Clinical, etc Notes
29	10/1/'17	7	—	—	—	—	—	—	0	0	1	1	0	0	0	0	0	0	mania
32	20/2/'17								0	0	0	1	1	0	0	0	0	0	? Tabes (per Dr Graham Brown)
36	20/3/'17	13	—	—	—	—	—	—	0	0	1	1	0	0	0	0	0	0	normal
37	20/3/'17	0	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	? G.P.I.
48	27/3/'17								0	0	0	1	1	0	0	0	0	0	? G.P.I. (per Dr Graham Brown)
53	6/4/'17	0 [R.B.C. a few]	—	—	—	—	—	—	0	0	0	1	1	0	0	0	0	0	normal (negative Cerebrospinal meningitis contact)
55	6/4/'17							—	0	0	0	1	0	0	0	0	1	1	? (not traced) (per Dr Graham Brown)
56	6/4/'17							—	0	0	0	0	1	1	1	1	0	0	Ditto
57	6/4/'17							—	0	0	0	0	1	1	1	1	0	0	Ditto
58	17/4/'17	0 [R.B.C. a few]	—	—	—	—	—	—	0	0	0	1	0	0	0	0	0	0	normal

Table (5) — Continued

No.	Date	Cells per c.mm.	Globulin Tests: Pándy	Noguchi	Ross-Jones	Nonne-Appelt	Wassermann Reaction: Serum	C-S.F.	Colloidal Gold Reaction: 1	2	3	4	5	6	7	8	9	10	Clinical etc Notes
59	17/4/17	0	—	—	—	—	+	—	0	0	0	1	1	1	1	0	0	0	Pos. blood W.R. & simulated G.P.I.
60	17/4/17	0	—	—	—	—	?	—	0	0	0	1	1	1	0	0	0	0	Normal
64	7/5/17	0	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	Normal
65	7/5/17	6	—	—	—	—	+	—	0	0	0	0	0	0	0	0	0	0	Lumbar puncture on suspicion, on account of pos. blood W.R.
66	7/5/17	8 [some blood contamination]	?	—	—	—		—	0	0	0	0	0	1	0	0	0	0	Normal
67	28/5/17	0 [R.B.C. a few]	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	Melancholia
68	28/5/17	2	—	—	—	—	+	—	0	0	0	0	0	0	0	0	0	0	Lumbar puncture on suspicion, on account of pos. blood W.R.
72	10/6/17	0	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	? Mental
79	27/6/17	4	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	Ditto.
85	6/8/17	0	—	—	—	—	+	—	0	0	0	0	0	0	0	0	0	0	Lumbar puncture on suspicion, on account of pos. blood W.R.

No	Date	Cells per c.mm.	Globulin Tests: Pándy	Noguchi	Ross-Jones	Nonne-Appelt	Wassermann Reaction: Serum	C.S.F.	Colloidal Gold Reaction: 1	2	3	4	5	6	7	8	9	10	Clinical, etc Notes
92	23/8/'17	20 [Some blood Contamination]	?	—	—	—	—	—	1	1	1	1	1	0	0	0	0	0	? G.P.I
93	23/8/'17	0	—	—	—	—	—	—	0	0	1	1	1	1	1	0	0	0	? mental
94	23/8/'17	2	?	—	—	—	+	—	0	0	0	0	1	1	1	0	0	0	Lumbar puncture on suspicion, on account of pos. blood W.R.
99	24/8/'17	4	—	—	—	—	+	—	0	0	0	1	1	1	0	0	0	0	Ditto.
102	10/9/'17	4	—	—	—	—	+	—	0	0	0	1	0	0	0	0	0	0	Ditto.
103	10/9/'17	1	—	—	—	—	—	—	0	0	0	0	1	0	0	0	0	0	Normal
104	10/9/'17	0	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	Normal
105	10/9/'17	2	?	—	—	—	+	—	0	0	0	0	0	0	0	0	0	0	Lumbar puncture on suspicion, on account of pos. blood W.R.
106	10/9/'17	2	—	—	—	—	—	—	0	0	0	1	0	0	0	0	0	0	Normal
108	10/9/'17	0 [Red Corpss. - a few]	Faint +	—	—	—	—	—	0	0	1	1	0	0	0	0	0	0	Normal

No	Date	Cells per c.mm.	Globulin Tests: Pándy	Noguchi	Ross–Jones	Nonne–Appelt	Wassermann Reaction: Serum	C.S.F.	Colloidal Gold Reaction: 1	2	3	4	5	6	7	8	9	10	Clinical, etc Notes
86	6/8/'17	0	—	—	—	—	+	—	0	0	0	0	0	0	0	0	0	0	Lumbar puncture on suspicion, on account of pos. blood W.R.
113	2/2/'19	0	—	—	—	—		—	0	0	0	0	0	0	0	0	0	0	Do. (Brighton V.D. Clinic)
116	17/2/'19	0	—	—	—	—		—	0	0	0	1	0	0	0	0	0	0	Ditto.
117	17/2/'19	4	—	—	—	—		—	0	0	0	0	0	0	0	0	0	0	Ditto.
119	19/2/'19	0	—					—	0	0	0	1	1	1	0	0	0	0	Ditto.
121	5/3/'19	0	—					—	0	0	0	0	0	0	0	0	0	0	Ditto.
122	5/3/'19	5	—					—	0	0	0	0	0	0	1	1	0	0	Ditto. (Brighton V.D. Clinic)

Table (5) — Concluded

APPENDIX II

Protocols of Precipitin Experiments

Protocols of "Globulin-Wassermann" experiments.

Protocols of Precipitin Experiments.

Date	Rabbit I 2 3 4	5 6 7 8
30/7/'17	each 2 cc. normal serum intravenously	each 2cc. Wassermann-reacting serum intrav.
2/8/'17	3-4 cc. intraperit.	3-4 cc. intraperit.
7/8/'17	ditto	ditto
10/8/'17	5-6 cc. intraperit.	5-6 cc. intraperit.
19/8/'17	ditto	ditto
1/9/'17	killed by bleeding	killed by bleeding

(Rabbit No. 7 received its third injection intravenously, and died with symptoms of anaphylactic shock.)

Sera sterilised with chloroform, subsequently evaporated at 37 deg., and tubed.

Nos. 3 and 8 were selected as giving the most abundant precipitum, and experiments set up of the following type:-

In each tube, 0.05 of the antiserum. A plus + denotes formation of precipitum.

	With normal antiserum				
dilutions of normal serum	+ 1:50	+ 1:100	+ 1:200	+ 1:400	? 1:800
dilutions of syphil. serum	+ 1:50	+ 1:100	+ 1:200	+ 1:400	− 1:800
dilutions of cerebro-spinal fluid (G.P.I.)	+ 1:5	+ 1:10	+ 1:20	+ 1:40	+ 1:80
dilutions of guinea-pig serum (control for human specificity)	− 1:50	− 1:100	− 1:200	− 1:400	− 1:800

normal serum	+ 1:50	+ 1:100	+ 1:200	+ 1:400	? 1:800
syphilitic serum	+ 1:50	+ 1:100	+ 1:200	+ 1:400	? 1:800
cerebrospinal fluid (G.P.I.)	+ 1:5	+ 1:10	+ 1:20	+ 1:40	+ 1:80
guinea-pig serum	− 1:50	− 1:100	− 1:200	− 1:400	− 1:800

Demonstration that the precipitin formed carries with it no indication as to whether the serum used in inducing its formation was normal or Wassermann-positive.

Also shown that neither of the sera has any complement-deviating power in the presence of tissue-extract or "antigen."

Protocols of "Globulin-Wassermann" experiments.

Preparation of normal and syphilitic globulins from the respective sera by precipitation and dialysis, as described in the text.

In each tube 0.1 cc. of solution of normal serum-globul.
In each tube 0.I cc. of "antigenic" emulsion (organ-extract plus cholesterin.)

No. of M.H.D. Complement. ⊖ ⊕ ⊕ ⊕ ⊕
1 2 4 6 8

Haemolysis denoted by plus +

In each tube 0.1 cc. of solution of Wassermann-positive serum-globulin.
In each tube 0.1 cc. "antigen".

No. of M.H.D. complement. ⊖ ⊖ ⊖ ⊖ ⊖
1 2 4 6 8

Demonstration that the Wassermann-reacting property of syphilitic sera resides in or is associated with the globulin fraction of the serum protein.